Gazi University Journal of Science
GU J Sci
24(4):657-662(2011)

Salinity Tolerance of Durum Wheat Genotypes in Androgenesis

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Received:06/06/2010 Revised: 24/11/2010 Accepted:27/02/2011

ABSTRACT

The aim of this study was to investigate the response of certain old and modern durum wheat genotypes (*Triticum durum* Desf. Kunduru 1149, Berkmen 469, Mirzabey, Altın and Yılmaz) to salinity using anther culture technique. Anther response and callus production were investigated in genotypes applying without and after cold pretreatments on spikes, and significant differences were found in the results. Berkmen 469 produced higher amount of calli both without and after cold pretreatment (7.47% and 16.42%, respectively) than other genotypes. The percentage of responsive anthers and production of calli increased after cold pretreatment in all genotypes tested in this study. Subsequently calli were transferred to the standard MS medium (control) and three different MS media containing various salt concentrations (NaCl doses of 0.5 mM, 1.0 mM and 1.5 mM). In this study, total plant regeneration was observed as 21.21% in Kunduru 1149 and 13.30% in Berkmen 469. Moreover, Kunduru 1149 succeeded in the plant regeneration on the MS medium modified by 0.5 mM and 1.0 mM NaCl concentrations.

Key words: *Triticum durum Desf., salinity, androgenesis, plant regeneration*

1. INTRODUCTION

Doubled haploid techniques are significant types of biotechnics for the production of new genotypes particularly in crop plants. Gametes have haploid number of chromosomes, and it is possible to produce haploid plants by using gametes or gametic organs in culture conditions. Haploid chromosome numbers can be doubled with colchicine treatment before gametophytic phase of the plants [1]. This situation creates many possibilities to get all the genes with desirable characters as homozygous in diploid phase in a short time.

Anther culture (androgenesis) is a tissue culture technique to obtain haploid embryo or embryo-like calli using immature pollens (microspores) in anthers cultivated on nutrition media. Mid-uninucleate microspores switch from gametophytic to somatic developmental stage on nutrition media in order to induce androgenesis. Generally, genotype response is highly depended on various physical treatments in culture conditions; therefore, it is important to increase the number of haploid embryos and plants [2-5].

On the other hand, plants are intoxicated by high levels of Na^+ and Cl^- ions in soils. These ions cause high osmotic pressure in soil, which reduces the water use efficiency of crops [6, 7]. There are two possible ways to prevent this situation: one is to improve soil preservation techniques, and the other is to produce new genotypes for cultivation in soils containing higher concentration of salt ions.

Durum wheat is one of the most important crop plants in Mediterranean countries, and some parts of this region have arid and semi-arid climates with salt problem. In this study,five Turkish durum wheat genotypes (*Triticum durum* Desf. Kunduru 1149, Berkmen 469, Mirzabey, Altın and Yılmaz) were used for anther culture. The main aim was to evaluate their capacity for callus production and plant regeneration and to asses the possibility of plant regeneration for those genotypes in different salt (NaCl) concentrations.

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2. EXPERIMENTAL

2.1. Materials

T.durum Desf. Kunduru 1149, Berkmen 469, Mirzabey, Altın and Yılmaz (2n=4x=28) were used as plant materials in this study. Kunduru 1149 and Berkmen 469 are old genotypes improved with the selection of landraces; on the other hand, Mirzabey, Altın and Yılmaz are improved as modern cultivars with different conventional breeding techniques. Seeds of genotypes were provided from Ankara Crop Plants Central Research Institute.

2.2. Methods

Cultivation of donor plants: Plants were grown in pots which contain peat and sand (3:1) in a plant growth room in tissue culture laboratory of Department of Biology. They were irrigated with tap water and supplemented with major and minor nutrients. Light was supplied by lamps (10-15 klux m^{-2}) on a photoperiod of 16h/8h day/night at 18 ± 2ºC.

The spikes for anther culture were collected when the microspores were at mid- or late-uninucleate stage. Inter-ligule length on top of the tiller was used as an indicator of this stage.

Cold pretreatment: For cold pretreatment, each selected spike surface was sterilized with 70% ethanol and kept in a refrigerator at 4°C for 7 days. Spikes were placed in 9 cm petri dishes containing several drops of sterile distilled water to maintain humidity, sealed with parafilm and then wrapped with aluminum foil. After a week, the spikes were selected according to developmental stage of microspores (in a drop of 2% acetocarmine solution, under the light microscope), and anthers were plated onto the media (Figure 1a) [5].

Preparation of media: Two different media were used for induction and regeneration. The anthers were plated onto 190-2, a liquid medium, developed for wheat by Wang and Hu (1984) containing 2 mg/l NAA and 1 mg/l Kinetin as an induction medium [8]. The media was prepared according to Savaskan et al. (1999) [5]. They were sterilized with a cellulase membrane filter (pore size is 0.22 µm) and added to autoclaved Ficoll 400. pH of the media was adjusted using 1N NaOH and 1N HCl to 6.2 and 5.8 for induction and regeneration media, respectively.

The regeneration medium was solidified by agar with 2 mg/l IAA and 1 mg/l Kinetin for standard MS control medium. Also, the regeneration medium was prepared by modifying three different amounts of NaCl (0.5, 1.0 and 1.5 mM per group).

Plating density of anthers on the induction medium was around 10-20 anthers per ml of medium (Figure 1b). For incubation, plated anthers were placed in an incubator at 28±1ºC in dark. Media were replenished by adding 1 ml of fresh medium in the second and forth weeks of incubation.

After 35 to 40d, calli were transferred onto regeneration medium in a 9 cm diameter petri dish when they reached 2-3 mm size (Figure 1c). Embryoids/calli were put in petri dishes and cultured under cool white fluorescent lamps at 22ºC with a 16h/8h photoperiod (Figure 1d). Photos were taken with a stereo microscope, Novex AP-8, and bars were determined using Microsoft Jawa, Image J.

Statistical evaluations: The number of responsive anthers was counted, and their percentage was calculated for each genotype, and also calli frequency was calculated per 100 anthers that were plated onto the liquid medium. Control of adaptation to anther culture technique of genotypes was performed using χ^2 (chi square) statistics for both results of anther response and calli production without and after cold pretreatments. The frequencies of green and albino plantlets were calculated per 100 calli transferred onto the control regeneration medium and each treatment group.

3. RESULTS

The response of durum wheat genotypes to androgenesis: In total, 1592 anthers were put on 190-2 medium in this study. Berkmen 469 cultivar displayed the highest anther responses of 43.10% and 51.42% without and after cold pretreatments, respectively (Table 1 and 2). Anthers of Mirzabey responded 45.78%, and other cultivars, Yılmaz, Kunduru 1149 and Altın responded 40.33%, 38.03% and 35. 29%, respectively, after a 7-day cold pretreatment, (Table 2) while Yılmaz didn't give any response to androgenesis without cold pretreatment in this study (Table 2). Kunduru 1149 didn't differ in anther responsiveness between without and after cold pretreatments, 33.45% and 38.03% respectively (Table 2). This genotype produced 9.41% calli after 7-day cold pretreatment, while the calli production was 5.03% without cold pretreatment (Table 1).

The adaptation of genotypes to culture conditions was controlled using χ^2 statistics. Genotypes didn't give the expected adaptation to anther culture in anther responsiveness without cold pretreatment, while the genotypes displayed adaptation with similar results after cold pretreatment at the 0.05 level (Table 2). In contrast, genotypes showed an consistency between the observed and expected values for calli production in anther culture, and it was important at the 0.05 level without cold pretreatment (Table 1). However, genotypes produced calli from anthers, significantly independent after a 7-day cold pretreatment (Table 2).

However, Kunduru 1149 produced the highest number of regenerant plant in this study (Table 3). Although the anther response and number of calli were obtained lower in Kunduru 1149 than Berkmen 469, they both produced the same number of calli to transfer to the regeneration media. The results of the responded anthers were found 38.03% and 51.42%; moreover, the calli production were 9.41% and 16.42% in Kunduru 1149 and Berkmen 469 genotypes, respectively (Table 1 and 2). In standard MS regeneration medium (control), Kunduru 1149 and Berkmen 469 produced

37.5% and 13.3% of total regenerant plants comparatively (Figure 1e and f) (Table 3 and 4).

Salinity tolerance of durum wheat genotypes in androgenesis: Kunduru 1149 produced 33 calli and transferred to the MS medium for plant regeneration. It produced regenerant plants in control and modified groups by NaCl concentrations (0.5 mM and 1.0 mM) (Table 3). However, green plants were observed in only one modified group containing 0.5 mM NaCl salt, except control (Figure 1f) (Table 3). Berkmen 469 also produced 6.7% green and 6.7% albino regenerant plants in control group in this study. On the other hand, there was no plant regeneration on the MS media modified by 0.5 mM, 1.0 mM and 1.5 mM concentrations of NaCl in Berkmen 469 (Table 4). In Mirzabey, Yılmaz and Altın, there was no calli to transfer to the regeneration medium in 2-3 mm size.

Table 1. The response of durum wheat cultivars to androgenesis without (0 day) cold pretreatment

Genotype	Number of anthers plated	Number of anthers responded	Number of calli produced	(%) Anther* responded (%)	Calli** production
Berkmen 469	174	75	13	43.10	7.47
Kunduru 1149	278	93	14	33.45	5.03
Yılmaz	140	22	6	15.71	4.28
Mirzabey	85	12	3	14.11	3.52
Altın	75	-	-	-	-
Total	752	202	36		

For anthers responded, $\chi^2 = 22.38$. There is no consistency between observed and expected values.
For calli production , $\chi^2 = 1.73$. The consistency between observed and expcted values is significant at the 0.05 probability level.
* The percentage of responsive anthers
** The frequency of calli/100 plated anthers

Table 2. The response of durum wheat cultivars to androgenesis after a 7-day cold pretreatment

Genotype	Number of anthers plated	Number of anther responded	Number of calli produced (%)	Anther* responded (%)	Calli** production
Berkmen 469	140	72	23	51.42	16.42
Kunduru 1149	255	97	24	38.03	9.41
Yılmaz	119	48	11	40.33	9.24
Mirzabey	190	87	16	45.78	8.42
Altın	136	48	3	35.29	2.20
Total	840	352	77		

For anther responded, $\chi^2 = 3.85$. The consistency between observed and expected values is significant at the 0.05 probability level.
For calli production, $\chi^2 = 11.15$. There was no consistency.
* The percentage of responsive anthers
** The frequency of calli/100 plated anthers

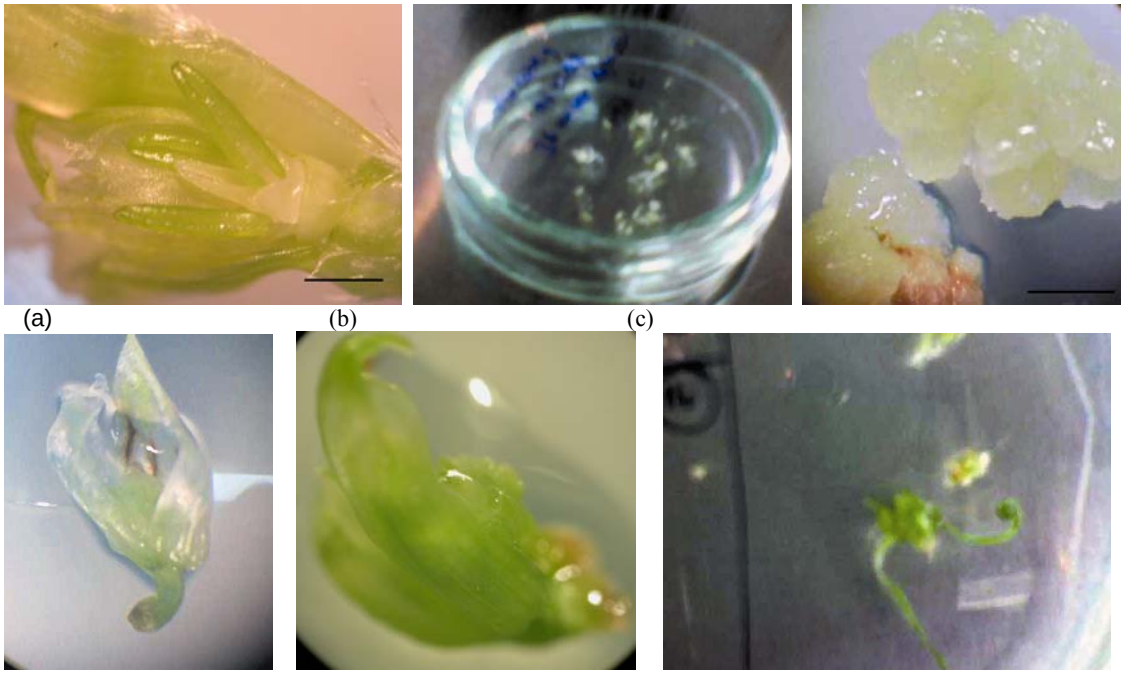

Figure 1. a) A durum wheat flower (*T.durum* Desf. cv. Berkmen 469), gynoecium in the middle and androecium (three anthers), b) anthers plated onto the 190-2 liquid medium, c) calli reached to the size of 2-3 mm, d) and e) albino and green regenerant plants from 'Berkmen 469' on standart MS medium and f) plant regeneration from 'Kunduru 1149' on MS+0.5 mM NaCl. (Bar=1000 µ)

Table 3. Plant regeneration of Kunduru 1149 in the standart MS medium and media modified by NaCl doses

Medium	Number of calli transferred (n)	Plant regeneration*		
		Green (%)	Albino (%)	Total (%)
MS control	8	12.5	25.0	37.5
MS+0.5 mM NaCl	8	12.5	12.5	25.0
MS+1.0 mM NaCl	10	-	20.0	20.0
MS+1.5 mM NaCl	7	-	-	-

*Frequency of plantlet/100 transferred calli

Table 4. Plant regeneration of Berkmen 469 in the standart MS medium and media modified by NaCl doses

Medium	Number of calli transferred (n)	Plant regeneration*		
		Green (%)	Albino (%)	Total (%)
MS control	15	6.7	6.7	13.3
MS+0.5 mM NaCl	6	-	-	-
MS+1.0 mM NaCl	6	-	-	-
MS+1.5 mM NaCl	6	-	-	-

*Frequency of plantlet/100 transferred calli

4. DISCUSSION

Many researchers stated that the success of androgenesis was due to the high genotypic effects, particularly in crop plants [2, 4, 5]. Also, Bregitzer (1992) reported significant responses to anther culture technique between *T. aestivum* and *T.turgidum* genotypes. According to their results, *aestivum* genotypes produced calli about 70.0%, while *turgidum*

genotypes produced half of this value (30-35%) in the same cultivar conditions [9].

Durum wheat genotypes also showed strong genetic dependence to androgenesis and were influenced by a 7-day cold pretreatment in both anther response and callus production in this study (Table 1 and 2). During the study, anthers of genotypes responded to androgenesis differently at the 0.05 probability level without cold pretreatment (Table 1). The highest responsive anthers and callus production were observed in Berkmen 469, 43.10% and 7.47% respectively (Table 1). Doğramacı-Altuntepe (2001) reported that the best responsive durum wheat genotype was Dicle-74, producing 1.28% calli in anther culture technique in their research with durum wheat genotypes [10]. Hassawi et al. (2005) found the highest amount of calli (3.6%) and green plant regeneration (1.78%) in Acsad-65, which is one of the Jordanian wheat genotype [11]. After cold pretreatment, the percentage of responsive anthers and the frequency of calli were found higher than without cold pretreatment in each genotype (Table 2). In the previous studies, the most effective pretreatment was found as the application of cold stress on spikes ranged from 72 hours to 4 weeks for wheat plants [12]. Savaskan et al. (1999) reported for the barley genotypes that the cold pretreatment created suitable physiological conditions to produce more calli and embryo like calli in genotype, and those were independent from genetic effects [5].

Kunduru 1149 had the least amount of anthers (38.03%) (Table 2). This genotype produced less amount of calli (9.41%) than Berkmen 469 after cold pretreatment in this study (Table 2). However, the former genotype produced the highest green and albino plantlets on regeneration media (Table 3). Berkmen 469 showed a good performance in the previous research related with intergeneric crosses producing haploid embryo in gynogenesis, and there was no statistically significant difference for Kunduru 1149. On the other hand, Kunduru 1149 showed better performance than Berkmen 469 for haploid plant regeneration in gynogenesis [1]. Berkmen 469 did not show enough genetic capacity to change the callus structure for the plant regeneration although it had a tendency to produce calli and/or embryoid calli in either androgenesis or gynogenesis.

Salinity is a major problem all over the world particularly in arid and semi arid regions. Because of the disadvantages of climatic problems and incorrect irrigation practices in agronomy, the soil structure has been deteriorated for a while. On the other hand, salt ions (Na^+) affect the resistance of the selective permeability of the root cell wall in plants if the soil has intolerable salt concentrations. This affects all the physiological and morphological structures of plants by vascular system from roots to all parts of the organism.

Durum wheats are difficult genotypes to produce calli and plant regeneration particularly in androgenesis conditions. There is a very limited number of studies on the salinity tolerance of durum wheat genotypes in culture media conditions, especially using anther cultures. Arzani and Mirodjagh (1999) had analysed the tolerance of 28 durum wheat varieties with various doses of NaCl in culture conditions by adopting their capacity using an immature embryo culture and callus production pathway [13]. They found that only two varieties (PI 40 100 and Dipper-6) gave better results than others.

In our study, calli produced from Kunduru 1149 and Berkmen 469 could be transferred to the MS control regeneration medium and treatment groups modified by 0.5 mM, 1.0 mM and 1.5 mM concentrations of NaCl. Both genotypes produced green and albino plantlets in control groups (Table 3 and 4). However, Kunduru 1149 also produced green and albino plantlets on MS modified by 0.5 mM NaCl and only an albino plantlet in the group modified by 1.0 mM NaCl (Table 3). Kunduru 1149 and Berkmen 469 are old durum genotypes selected from landraces and have large adaptation capacity to the environmental conditions because of their heterogeneous / heterozygote structures. This may be the reason for the better results they gave in androgenesis. Particularly, Kunduru 1149 succeeded to produce at least a small number of plantlets in the media with low salt concentrations than modern cultivars Mirzabey, Yılmaz and Altın.

ACKNOWLEDGEMENT

This study has been sponsored by BAP Project of Süleyman Demirel University (Project No: 06-YL-1250) and Turkish Scientific and Technological Reseach Authority (TÜBİTAK, Project No: TBAG 107T270, HD-303).

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